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Trimethyltin Impairs Retention of a Passive Avoidance Task[1]

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WALSH, T. J., M. GALLAGHER, E. BOSTOCK AND R. S. DYER. *Trimethyltin impairs retention of a passive avoidance task*. NEUROBEHAV. TOXICOL. TERATOL. 4(2) 163–167, 1982.—Trimethyltin is a neurotoxic organometal which produces neuronal damage in several limbic regions including the hippocampus, amygdala and the pyriform cortex. One administration of trimethyltin (5, 6 or 7 mg/kg) twenty one days prior to passive avoidance conditioning produced an impairment of retention when animals were tested 24 hours after training. Rats treated with trimethyltin exhibited shorter step-through latencies and freezing durations during the retention test. It was observed that the three dosages of trimethyltin were equally effective in disrupting retention performance. These retention deficits were not secondary to alterations in footshock sensitivity. The data presented here indicate that acute trimethyltin administration disrupted learning and memory. This compound might be a useful tool for examining the role of the limbic system in associative processes.

Trimethyltin Memory Organotins Neurotoxicology Limbic system Passive avoidance

ALKYLTINS are used for a variety of industrial and agricultural purposes such as polymer stabilization and pest control [21,22]. Their increasing use and potential health-related effects has prompted our laboratory to investigate their neurobehavioral toxicity.

Trimethyltin (TMT) is a common intermediate and byproduct in the synthesis of the more commonly used alkyltins [6,14]. This compound has been implicated in several episodes of human poisoning and has also been shown to produce a complex spectrum of both neuropathological and behavioral changes in laboratory animals. For example, acute or chronic exposure to TMT in the rat produces a relatively selective pattern of limbic system damage involving the hippocampus, amygdala and pyriform cortex [1,2]. Furthermore, pyramidal neurons in the CA3 cell field of the hippocampus appear to be particularly vulnerable to the toxic effects of TMT [4]. Acute administration of TMT also produces a transient behavioral syndrome consisting of anorexia, spontaneous seizures, self-mutilation and hyperexcitability [5]. While these behavioral effects are typically gone within fourteen days of dosing, recent work in our laboratory has characterized more subtle and persistent neurobehavioral consequences of TMT exposure (see [17,20]).

Accidental exposure to TMT in the workplace has been reported to produce a neurologic syndrome consisting of spontaneous seizures, emotional lability, anorexia and learning and memory impairments [8,16]. Since cognitive deficits were prominent symptoms in both of these incidents we were interested in whether TMT impaired memory processes. This hypothesis was supported by previous reports on the adverse consequences of limbic system damage on learning and memory [11,15].

In the first experiment we examined the dose-dependent effects of TMT on retention of a one-trial passive avoidance response.

EXPERIMENT 1

METHOD

Subjects

Forty-two male Long-Evans hooded rats obtained from Charles River Breeders (Wilmington, MA) were used in the present study. All rats were individually housed in hanging metal cages in a temperature and humidity controlled animal colony under a 12 hr/12 hr light-dark cycle. Laboratory chow and tap water were freely available throughout the experiment. At the start of the experiment the animals were 90–120 days of age and weighed between 250–300 grams.

[1]This manuscript has been reviewed by the Health Effects Research Laboratory, U. S. Environmental Protection Agency and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. Send reprint requests to first author.

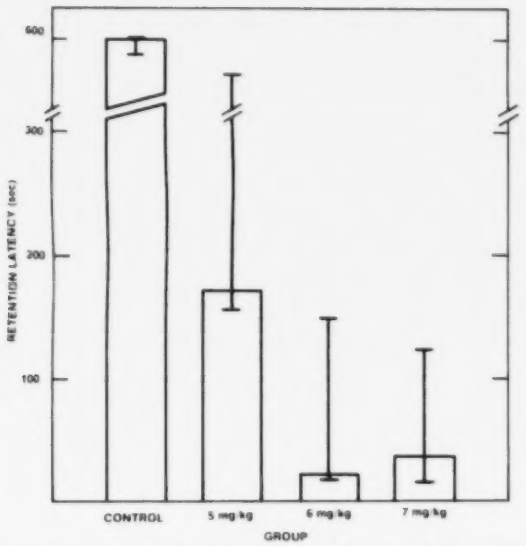

FIG. 1. Effects of 5 mg/kg (n=8), 6 mg/kg (n=11) or 7 mg/kg (n=6) of trimethyltin chloride on 24 hour retention latencies in a step-through passive avoidance task. Bars represent the median retention latencies with interquartile ranges. The control group represents the pooled data of the saline injected (n=8) and non-injected controls (n=9); an independent two-tailed Mann-Whitney U test revealed no significant differences between these groups ($p>0.10$). Two-tailed Mann-Whitney U tests performed on the experimental groups revealed significantly shorter latencies for the 5 mg/kg ($p<0.02$), 6 mg/kg ($p<0.002$) and 7 mg/kg ($p<0.002$) groups compared to the control group.

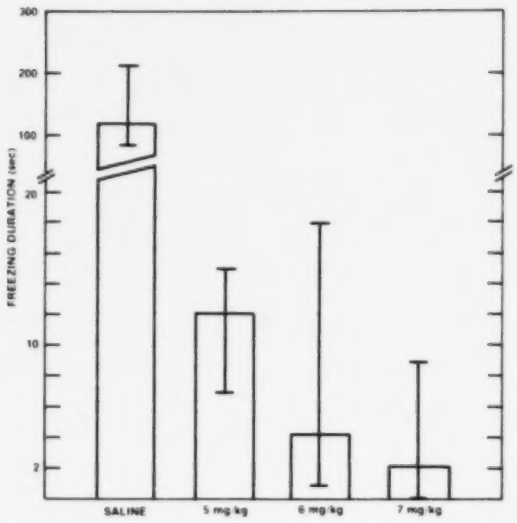

FIG. 2. Effects of trimethyltin chloride on freezing durations during the retention test. Bars represent median freezing durations with interquartile ranges. Independent two-tailed Mann-Whitney U tests revealed significantly shorter freezing durations for the 5 mg/kg ($p<0.003$), 6 mg/kg ($p<0.05$) and 7 mg/kg ($p<0.001$) groups as compared to the controls.

Treatment

Rats were intragastrically intubated with either physiological saline (vehicle, n=8), 5 (n=8), 6 (n=11) or 7 (n=6) mg/kg TMT Cl (dosage calculated as base of TMT). Dosing occurred twenty one days prior to passive avoidance training. This interval was chosen to insure that neuropathological changes would be fully manifest (see [2]) and the acute toxic effects of TMT dissipated [5] by the time of training. A non-intubated control group (n=9) was also included. TMT Cl was obtained from ICN Pharmaceuticals (Plainview, NY) and administered in a volume of 1 ml/kg body weight.

Apparatus

A step-through passive avoidance apparatus similar to one introduced by Jarvik and Kopp [12] was used. The apparatus consisted of a V-shaped trough which was divided by a guillotine door into a small illuminated start box and a larger darkened box. Stainless steel panels formed the walls and floor of the trough and served, in the dark box, to deliver AC shock to the animal's feet. The shock generator was a Coulbourn Instruments Model E13-16 Constant Current Solid State Shocker.

Procedure

On each of three days prior to training the rats were individually handled for approximately 3 min. A pre-training session, in which the rat was placed into the apparatus and removed immediately following a step-through response occurred 24 hours prior to training. During this pre-training session and the subsequent training trial the guillotine door remained open. Twenty-four hours later each rat was trained in a single trial passive avoidance task. Passive avoidance conditioning consisted of placing the rat into the illuminated start chamber and recording the latency to step-through into the darkened section. Immediately following step-through (defined as the passage of the hind limbs beyond the threshold) the door was closed and the rat given a 0.6 mA footshock for 1 sec. The retention test was performed 24 hours later during which the rat was again placed into the start box and the step-through latency recorded to an arbitrary maximum of 600 sec. The step-through latency served as the dependent measure and was taken to indicate the degree of retention of the task. The duration of time the animal remained immobile following placement into the apparatus during the retention test was also measured (i.e., freezing duration).

RESULTS

Some of the animals treated with 6 or 7 mg/kg TMT exhibited transient weight loss and hyperexcitability. By the time of training however, they were generally comparable to the controls.

A Kruskal-Wallis nonparametric analysis of variance demonstrated a significant treatment effect on initial step-through latencies during the training trial, $H(4)=12.1$, $p<0.02$. Post-hoc group by group comparisons with a two-tailed Mann-Whitney U test demonstrated that the latency of the 7 mg/kg group was significantly longer than the latencies of the other groups ($p<0.005$). There were no other significant between-group differences. The median initial step-

through latencies of the 0, 5, 6 and 7 mg/kg groups were 19, 16, 21 and 68 sec, respectively.

The data from the first experiment revealed that acute administration of TMT produced impaired retention of a single-trial passive avoidance task (see Figs. 1 and 2). A Kruskal-Wallis nonparametric analysis of variance demonstrated a significant treatment effect on both step-through latencies, $H(4)=24.57$, $p<0.001$, and freezing durations, $H(4)=16.3$, $p<0.01$, during the retention test. As can be seen in the figures the retention impairment was coincident with shorter freezing durations in the treated animals.

While the TMT-treated rats exhibited poor retention of the task, this was not a dose-related phenomenon. A Kruskal-Wallis analysis of variance performed on the step-through latencies and freezing durations of the groups treated with TMT revealed no significant between-group differences on these measures, $H(2)=5.04$, $p>0.05$, for step-through latencies; $H(2)=3.59$, $p>0.05$, for freezing durations. Therefore, 5, 6 or 7 mg/kg TMT produced comparable deficits in passive avoidance retention.

In the second experiment we examined whether the retention impairments were secondary to alterations in reactivity to footshock.

EXPERIMENT 2

TMT has been reported to alter the responsiveness of several sensory systems. For example, acute TMT alters the visual-evoked potential [3] and attenuates the acoustic startle response [9]. Of greater relevance to the interpretation of the results of the first experiment is the report that TMT compromises behavioral reactivity to noxious stimuli. We recently reported that acute TMT administration produced time-dependent changes in hot-plate latencies indicative of somatosensory dysfunction [10]. Taken together, these reports could indicate that TMT changes sensitivity to sensory stimulation in general. If the TMT-treated rats were less sensitive to the footshock during the training session, then the resulting retention deficits might be secondary to changes in perception of footshock and not to disrupted memory processes. To investigate this possibility we examined the sensitivity of TMT-treated rats to electric footshock using a modified Evans [7] flinch-jump procedure.

METHOD

Subjects

The rats described for Experiment 1 were retested in this experiment.

Apparatus

The apparatus was a transparent Plexiglas operant chanber with a grid floor. The maniplulanda had been removed prior to the start of the experiment. Scrambled AC footshock was delivered to the grid floor by a Coulbourn Instruments Solid State Shocker identical to the one used in Experiment 1 for avoidance training.

Procedure

Approximately 1 week following the retention test, the jump thresholds were determined for the rats used in Experiment 1. At the beginning of testing each rat was allowed a 60 sec shock-free period to acclimate to the apparatus. Following this period, six series of unavoidable footshocks were presented in alternating ascending and descending intensities. The shock generator was set at 0.05, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80 or 0.90 mA. Footshock duration was 1 sec and there was a 15 sec interstimulus interval. A 60 sec shock-free period was allowed between alternating series. Shock presentation in the first series was always in ascending order.

The presence or absence of a jump response, defined as the simultaneous removal of all 4 paws from the grid floor, was determined at each shock intensity. The threshold for each animal was computed as the mean shock level at which a jump response was reliably recorded. In the present experiment we chose not to measure flinch thresholds since we were primarily interested in how the treated rats reacted to footshock intensities comparable to those used in Experiment 1 (i.e., 0.6 mA for 1 sec).

TABLE 1

MEDIAN JUMP THRESHOLDS OF RATS TREATED WITH 0, 5, 6 OR 7 mg/kg TMT Cl

Group	n	Jump Threshold (mA)	Interquartile Range
Saline	8	0.36	0.33–0.41
5 mg/kg TMT	8	0.41	0.33–0.47
6 mg/kg TMT	11	0.42	0.28–0.45
7 mg/kg TMT	5	0.48	*

*Interquartile range not computed due to the small experimental n.

RESULTS

The rats treated with TMT had jump thresholds which were comparable to those of the controls. The median jump thresholds for the groups treated with 0, 5, 6 or 7 mg/kg TMT are presented in Table 1. A Kruskal-Wallis nonparametric analysis of variance demonstrated that there were no significant differences between groups on this measure, $H(3)=3.44$, $p>0.10$. These data indicate that TMT did not impair responsiveness to footshock, as assessed by this method, and further suggest that the retention deficits observed in Experiment 1 were not secondary to changes in footshock sensitivity.

GENERAL DISCUSSION

The results of the first experiment demonstrated that TMT produced an impairment of retention for a one-trial shock-motivated passive avoidance task. The groups administered 5, 6 or 7 mg/kg TMT twenty one days prior to training had shorter step-through latencies and freezing durations than the controls during the 24 hour retention test. While these data suggest that TMT impaired either the initial acquisition or consolidation of the task several other alternatives should be considered. For example, it could be argued that the acute toxic effects of TMT, which include weight loss and hyperexcitability indirectly contributed to the shorter step-through latencies during the retention test. It was observed however, that at the time of training (i.e., 21 days after dosing) there were no differences in body weight or apparent difficulty of handling between groups. Furthermore, the group administered 5 mg/kg never did lose weight

or exhibit enhanced excitability although they were impaired in retaining the task. Therefore, it is unlikely that the retention impairments seen in Experiment 1 were secondary to the nonspecific toxicity of TMT. It was observed during training however, that the 7 mg/kg TMT group had longer initial step-through latencies than any of the other groups. This could indicate that toxicant-induced alterations in locomotor activity, attention or emotionality might have contributed, in some unspecified way, to the retention deficits seen in this group. Since the other TMT-treated groups did not exhibit longer training latencies than the controls however, this explanation cannot serve as a global interpretation of TMT's effects on passive avoidance retention.

The second experiment demonstrated that rats treated with TMT were behaviorally just as reactive to footshock as the controls. There were no differences between any of the groups in jump thresholds following presentation of a series of unavoidable footshocks. Caution must be exercised in extending the generality of these results because the physical characteristics of the chambers (i.e., dimensions, surface area, grids vs metal plates, etc.) in which shock was delivered in Experiments 1 and 2 were different. While the lack of a TMT effect on shock sensitivity might seem contrary to previous results from our laboratory demonstrating time-dependent increases in hot-plate latencies following TMT, several explanations might be offered to account for the apparent discrepancies. For example, the jump-flinch procedure and the hot-plate test might measure behavioral responses which are organized at different levels of the neuraxis. The jump-flinch procedure seems to assess spinally-mediated reflexes whereas the behavioral response to the hot-plate could be mediated by a more central integrative process. Our data indirectly support this hypothesis since we observed no differences in peripheral nerve function following TMT but did find changes in central somatosensory processes [10]. Another alternative is that TMT produced alterations in neuromotor function which confounded the analysis of pain sensitivity. Tilson and Burne [19] reported that triethyltin-induced changes in pain sensitivity were the consequence of neuromotor impairment rather than altered behavioral reactivity to noxious stimuli. While TMT-treated rats exhibit tremor which might contribute to impaired neuromotor capacity this effect is seen only with higher doses and is typically gone within ten to fourteen days. This time course might coincide with the changes in hot-plate latencies observed four days after dosing and with the apparent lack of effect on shock sensitivity thirty five days following dosing. In light of the apparent discrepancies a more complete characterization of the TMT-induced changes in behavioral and electrophysiological correlates of pain sensitivity is warranted. For the purpose of interpreting the present experiment, however, it is clear that the treated animals were as reactive to the footshock as the controls. Therefore, it is reasonable to conclude that TMT administration altered retention of the task by disrupting neural processes or events which participate in learning and memory.

While histology was not performed on the animals used in these experiments several comments on the time course and characteristics of the neuropathology associated with similar dosing regimens seem appropriate. Brown and coworkers [2] observed cell loss in the hippocampus within ten days of a single 10 mg/kg dose of TMT. Maximal neural damage, evident at 21 days after dosing, was characterized by extensive cell loss in the hippocampus, pyriform cortex, dentate gyrus and amygdala. More recently, Dyer and colleagues [4] reported a dose-related decrease in both the length of the pyramidal cell line and the thickness of the dentate gyrus 30 days following a single dose of 5, 6 or 7 mg/kg or TMT. Furthermore, administration of either of the higher doses reduced the density of cells in the CA3 portion of the pyramidal cell line. While these reports have established the preferential sensitivity of limbic forebrain regions to TMT-induced neurotoxicity, it will be important for future studies to correlate the neuropathology with the behavioral changes.

In conclusion, the experiments reported here demonstrated that acutely administered TMT produces retention impairments for a passive avoidance task. This observation is consistent with the extensive literature on the adverse effects of limbic system damage on avoidance learning. For example, Isaacson [11] reported that lesions of either the amygdala or the hippocampus were capable of affecting aversively-motivated learning in a variety of test situations. Since TMT produces a relatively selective limbic system toxicity it might be used to investigate the physiology and function of this neural system. Furthermore, recent evidence indicates that TMT might be a particularly useful tool for elucidating the neurobiological substrates of memory processes. For example, it has been reported that TMT impairs the acquisition and performance of complex spatial tasks such as the radial-arm maze [20] and the Hebb-Williams maze [18] and also disrupts retention of two-way [13] and passive avoidance (present data) responses. Therefore, studies examining the biochemical and neural correlates of TMT-induced behavioral changes might provide further insights into the biology of learning and memory.

REFERENCES

1. Bouldin, T. W., N. D. Goines, C. R. Bagnell and M. R. Krigman. Pathogenesis of trimethyltin neuronal toxicity: Ultrastructural and cytochemical observations. *Am. J. Path.* **104**: 237–249, 1981.
2. Brown, A. W., W. N. Aldridge, B. W. Street and R. D. Verschoyle. The behavioral and neuropathologic sequelae of intoxication by trimethyltin compounds in the rat. *Am. J. Path.* **97**: 59–82, 1979.
3. Dyer, R. S., W. E. Howell and W. F. Wonderlin. Visual system dysfunction following acute trimethyltin exposure in rats. *Neurobehav. Toxicol. Teratol.* **4**: 191–195, 1982.
4. Dyer, R. S., T. L. Deshields and W. F. Wonderlin. Trimethyltin-induced changes in gross morphology of the hippocampus. *Neurobehav. Toxicol. Teratol.* **4**: 141–147, 1982.
5. Dyer, R. S., T. J. Walsh, W. F. Wonderlin and M. Bercegeay. The trimethyltin syndrome in rats. *Neurobehav. Toxciol. Teratol.* **4**: 127–133, 1982.
6. EPA Report No. 560/-6-76-011. *The Manufacture and Use of Selected Alkyltin Compounds*. 1976.
7. Evans, W. O. A new technique for the investigation of some analgesic drugs on a reflexive behavior in the rat. *Psychopharmacologia* **2**: 318–325, 1961.
8. Fortemps, E. G., Amand, A. Bomboir, R. Lauwerys and E. C. Laterre. Trimethyltin poisoning: Report of two cases. *Int. Archs occup. Envir. Hlth* **41**: 1–6, 1978.
9. Howell, W. E., R. S. Dyer, W. F. Wonderlin, K. Kidd and L. W. Reiter. Sensory system effects of acute trimethyltin exposure. *Toxicologist* **1**: 43–44, 1981.
10. Howell, W. E., T. J. Walsh and R. S. Dyer. Somatosensory dysfunction following acute trimethyltin exposure. *Neurobehav. Toxicol. Teratol.* **4**: 197–201, 1982.
11. Isaacson, R. L. *The Limbic System*. New York: Plenum Press, 1974.

12. Jarvik, M. E. and R. Kopp. An improved one-trial passive avoidance learning situation. *Psychol. Rep.* **21**: 221–224, 1967.
13. Mactutus, C. F., J. J. Valdes, J. S. Young and Z. Annau. Trimethyltin neurotoxicity: A potential tool for learning and memory. *Soc. Neurosci. Abstr.* **7**: 647, 1981.
14. National Institute of Occupational Safety and Health. *Criterion for a Recommended Standard: Occupational Exposure to Organotins*. Washington, DC: U. S. Government Printing Office, 1976.
15. O'Keefe, J. and L. Nadel. *The Hippocampus as a Cognitive Map*. London: Oxford University Press, 1978.
16. Ross, W. D., E. A. Emmett, J. Steiner and R. Tureen. Neurotoxic effects of occupational exposure to organotins. *Am. J. Psychiat.* **138**: 1092–1095, 1981.
17. Ruppert, P. H., T. J. Walsh, L. W. Reiter and R. S. Dyer. Trimethyltin-induced hyperactivity: Time course and pattern. *Neurobehav. Toxicol. Teratol.* **4**: 135–139, 1982.
18. Swartzwelder, H. S., J. Hepler, W. Holahan, S. E. King, H. A. Leverenz, P. A. Miller and R. D. Myers. Severely impaired maze performance in the rat caused by trimethyltin treatment: Problem-solving deficits and perseveration. *Neurobehav. Toxicol. Teratol.* **4**: 169–176, 1982.
19. Tilson, H. A. and T. A. Burne. Effects of triethyltin on pain reactivity and neuromotor function of rats. *J. Toxicol. Envir. Hlth* **8**: 317–324, 1981.
20. Walsh, T. J., D. B. Miller and R. S. Dyer. Trimethyltin, a selective limbic system neurotoxicant, impairs radial-arm maze performance. *Neurobehav. Toxicol. Teratol.* **4**: 177–183, 1982.
21. Watanabe, I. Organotins (Triethyltin). In: *Experimental and Clinical Neurotoxicology*, edited by P. S. Spencer and H. S. Schaumberg. Baltimore: Williams and Wilkins, 1980, pp. 545–557.
22. World Health Organization. *Tin and Organotin Compounds: A Preliminary Review*. Geneva: WHO, 1980.